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Table of Contents

Cover.....	
SF 298.....	
Table of Contents.....	
Introduction.....	3
Body.....	4
Key Research Accomplishments.....	5
Reportable Outcomes.....	6
Conclusions.....	6
References.....	6
Appendices.....	6

Introduction

Prostate cancer is the most common malignancy and is the second leading cause of cancer death among males in the United States (1). The development of prostate cancer is initially androgen-dependent (2). Therefore, androgen ablation therapy, which results in programmed cell death (apoptosis) in androgen-dependent prostate cancer cells, has been the current choice to treat prostate cancer (2). However, malignant prostate cancer cells often recur and are able to proliferate in an androgen-independent manner (3). Furthermore, androgen-independent prostate cancer cells are not only refractory to hormonal therapy, but also resistant to conventional chemotherapy and radiation treatment (4). Although the molecular mechanism of this resistance is still elusive, it may result from dysregulation in cell cycle control and the intracellular survival/death signaling pathways (5-14).

A fine balance between cell survival and death signaling pathways dictates proliferation or death of normal or malignant cells. In prostate cancer cells, expression level of bcl-2, a key anti-apoptotic protein, and the activity of NF- κ B, which is a transcription factor that suppresses apoptosis in many types of cells, were upregulated (10-18). These changes may allow malignant prostate cancer cells to escape from death. Consistently, prostate cancer cells were found to be less sensitive to death insults such as tumor necrosis factor (TNF)- α (15) or γ -irradiation (19, 20). It appears that prostate cancer cells may have acquired higher capacity for survival. If this is true, suppression of the cellular surviving signaling machinery should sensitize prostate cancer cells to death insults. **Investigation of signaling mechanisms underlying the growth of malignant prostate cancer cells, therefore, might provide a novel approach for prevention and treatment of the disease, and identify potential therapeutic targets for the prostate cancer therapy.**

In this proposal, we postulate to investigate the role of the cellular surviving signaling machinery in androgen-independent growth of prostate cancer cells and in their resistance to conventional therapy, using the I κ B kinase (IKK) pathway as a model system.

OBJECTIVES: **Specific Aim #1:** To determine the role of the IKK signaling pathway in the development of androgen-independent growth of prostate cancer cells
Specific Aim #2: To determine the role of the IKK signaling pathway in apoptosis-resistance of prostate cancer cells

BODY

Statement of Work

Signaling Mechanisms of Malignant Growth of Prostate Cancer Cells

Task 1: Investigation of the role of the IKK signaling pathway in the development of androgen-independent growth of prostate cancer cells, using androgen-dependent LNCaP 104-S and androgen-independent LNCaP 104-R cells: Month 1-24.

- a. *To determine expression level of endogenous NF- κ B proteins and the IKK complex by immunoblotting analysis.*

We have found that there is no difference in expression level of IKK and p65/NF- κ B in LNCaP 104-S and 104-R cells. This suggests that if there is a differential regulation of the IKK signaling pathway in 104-S and 104-R cells, it will be a post-translational mechanism.

- b. *To determine NF- κ B DNA-binding activity by electrophoresis mobility shift assays, I κ B phosphorylation and degradation by immunoblotting analysis, and the IKK complex activity by immunokinase assays.*

We found that there are no significant difference in basal level of activity of IKK and NF- κ B in 104-S and 104-R cells. This suggests that the IKK signaling pathway may play a role that is necessary but not sufficient in 104-R cells

- c. *To determine the induction of NF- κ B and the IKK complex by death insults such as TNF- α and γ -radiation.*

We found that induction of NF- κ B and the IKK complex by TNF- α were similar in both 104-S and 104-R cells. This suggests that if the IKK signaling pathway is involved in androgen-independent growth and apoptosis-resistance of 104-R cells, it may be necessary but not sufficient. The γ -radiation experiment is in progress.

- d. *To determine the impact of inhibition of the IKK signaling pathway on androgen-independent growth of LNCaP 104-S cells by stable expression or recombinant adenovirus of the dominant negative IKK mutant.*

Stable cell lines that expressing the dominant negative IKK β mutant are being established and will be characterized. The recombinant adenovirus of the DN IKK β mutant has been produced. We are in the process to determine whether the IKK signaling pathway is necessary for 104-R cells survival.

- e. To prepare manuscripts.

We expect to submit a manuscript describing our novel finding in year 2.

Task 2: Investigation of the role of the IKK signaling pathway in apoptosis-resistance of prostate cancer cells, Month 13-36

- f. To characterize recombinant dominant IKK β mutant, Ad/IKK β (SS \rightarrow AA), and the constitutively activated IKK β mutant, Ad/IKK β (SS \rightarrow EE), in prostate cancer cells (LNCaP, DU-145, and PC-3).

Both active and dominant negative IKK β mutants have been amplified and tested.

- g. To determine whether activation of the IKK signaling pathway by Ad/IKK β (SS \rightarrow EE) promotes LNCaP 104-S cells (androgen-dependent) to proliferate in androgen-privation conditions and protects the cells from apoptosis induced by TNF- α , γ -radiation, or chemodrugs.

We have started to test these hypotheses.

- h. To determine whether inhibition of the IKK signaling pathway by Ad/IKK β (SS \rightarrow AA) sensitizes androgen-independent prostate cancer cells to apoptosis induced by TNF- α , γ -radiation, or chemodrugs.

These have been initiated and in progress.

- i. To determine whether inhibition of the IKK signaling pathway by Ad/IKK β (SS \rightarrow AA) suppresses androgen-independent prostate cancer cells to form tumors in athymic mice.

These have been initiated and in progress.

- j. To prepare manuscripts and final reports.

KEY RESEARCH ACCOMPLISHMENTS

- a. Determine there is no significant difference in basal and activation of IKK and NF- κ B in 104-S and 104-R cells.
- b. Preparation of adenovirus and stable cell lines that harbor the IKK mutants.

Anning Lin, Ph.D.

REPORTABLE OUTCOMES

Invited Speaker, Co-Chair of the mini-symposium of "Defining Genes and Mechanisms Regulating Metastasis", American Association for Cancer Research Annual meeting, March 24-28, 2001, New Orleans, LA. The title of the talk is "The role of the IKK complex in apoptosis and metastasis of prostate cancer".

CONCLUSIONS

Our preliminary results indicate that the IKK signaling pathway may be necessary but not sufficient for androgen-independent growth and apoptosis-resistance.

REFERENCE

NONE

APPENDICES

NONE